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Plasma Catecholamine and Corticosterone Levels During Active and Passive Shock-Prod Avoidance Behavior in Rats: Effects of Chlordiazepoxide[1]

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DE BOER, S. F., J. L. SLANGEN AND J. VAN DER GUGTEN. *Plasma catecholamine and corticosterone levels during active and passive shock-prod avoidance behavior in rats: Effects of chlordiazepoxide.* PHYSIOL BEHAV **47**(6) 1089–1098, 1990.—Plasma noradrenaline (NA), adrenaline (A) and corticosterone (CS) concentrations were determined in rats before, during and after 15-min exposure to a constantly electrified (2 mA) or nonelectrified prod which was mounted on the wall of the home cage either with or without bedding material on the floor. Concomitantly, exploration of the prod, freezing and prod-burying behavior were recorded. Both in the presence and absence of bedding material, rats explored the nonelectrified prod and showed a small increase in plasma NA and CS contents. Exploration of the prod was strongly reduced when the prod was electrified. In the presence of bedding material, shocked rats typically displayed burying behavior (active avoidance), whereas in the absence of bedding (i.e., burying option eliminated) shocked rats engaged in freezing behavior (passive avoidance). The passive avoidance situation was accompanied by larger A and CS increases but a lower NA rise as compared to the hormonal responses associated with the active avoidance situation. Administration of the anxiolytic chlordiazepoxide (CDP; 9 mg/kg intragastrically) attenuated the shock-induced suppression of prod exploration, decreased prod-burying behavior but, paradoxically, increased freezing behavior. Irrespective of bedding condition, the prod shock-induced elevations in plasma CS and A contents were completely abolished in CDP-treated rats. The rise in plasma NA was attenuated only in CDP-treated rats tested on a bedding-floor. The results indicate that passive (e.g., freezing) and active (e.g., burying) behavioral coping are each accompanied by specific and dissociated patterns of neurosympathetic, adrenomedullary and adrenocortical outflow. CDP-treatment shifts an animal's behavioral coping style from an active to a passive form of avoidance responding, but abolishes the accompanying adrenocortical and adrenomedullary activation.

Noradrenaline Adrenaline Corticosterone Stress Defensive burying Freezing Coping Chlordiazepoxide Rat

RATS display different behavioral response styles when coping with adverse environmental events. One type of response is characterized by immobility and suppression of environmentally directed activities, i.e., the passive, conservation-withdrawal mode of response (5, 12, 17). This is in contrast with the other response style which is characterized by active responding whereby the animal displays much locomotor activity in attempting to escape from or to deal with an external threat, i.e., the fight/flight defense pattern of reaction (5, 8, 17). It has been claimed that the neuroendocrinological consequences of these behavioral strategies differ as well: the passive mode of response being attended by a predominant activation of the pituitary-adrenocortical axis which results in raised plasma concentrations of the glucocorticoid corticosterone (CS), whereas the active mode of response is accompanied by a preferential sympathetic-adrenomedullary activation, resulting in an increased release of noradrenaline (NA) and adrenaline (A) into the blood stream (8, 14, 17, 18, 22). The balance between these two modes of stress response seems to depend on the animal's predisposition toward one of the two strategies and the degree of factual or perceived behavioral control over the aversive stimulus situation (4, 13, 15, 17).

This concept of distinctive patterns of neuroendocrine stress responses is based almost exclusively on results from a number of human and animal experiments performed within a social context (13, 14, 18). The generality of the model has not yet been validated in nonsocial situations. In the traditional shock avoidance/escape paradigms which have been used to study the neuroendocrinological correlates of coping behavior, a differentiation

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between catecholamine (CA) and CS responses has not been demonstrated. Escapable as well as yoked inescapable shocked rats responded with a simultaneous activation of the pituitary adrenocortical axis and the sympathetic adrenomedullary systems; the magnitude of the ensuing increase in plasma CS, NA and A concentrations being similarly dependent upon the ability or inability of the animal to control the shock-stressor (2, 11, 29, 37). This finding may be explained by the fact that these experimental settings impose serious restrictions on the rat's ability to perform species-typical defensive behavioral responses in order to deal effectively with the aversive stimulus (5, 26, 31, 40): e.g., most of the paradigms require that coping rats have to perform a demanding and unnatural operant task (lever pressing) in order to escape or avoid a diffuse electric shock. Moreover, the traditional paradigms usually require multiple conditioning sessions as well as extensive handling and/or transfer procedures which might easily disturb the neuroendocrine systems under study (7,19).

Recently, another avoidance paradigm without these constraints has been described. Pinel, Treit and co-workers (26,31) reported that when suitable bedding material is available, rats have a strong innate tendency to bury a well-localized source of noxious stimulation, such as an electrified prod mounted on the wall of the chamber from which they have received electric shock. Burying of the electrified prod represents an active form of avoidance behavior (32, 34, 39). However, in an environment without bedding material, where the burying option is eliminated, rats have been reported to display a passive form of avoidance behavior, e.g., freezing in locations away from the prod (32). Therefore, this so-called shock-prod/defensive burying paradigm might be a convenient test situation to study the neuroendocrine correlates of active and passive avoidance behavior in rats.

Accordingly, the purpose of the present study was to determine the plasma NA, A and CS changes accompanying shock-prod defensive burying and freezing behavior. In addition, the effects of the prototypical anxiolytic agent chlordiazepoxide (CDP) were assessed on the shock-prod-induced behavioral and accompanying hormonal responses. A number of studies have shown that benzodiazepine (BDZ) anxiolytic drugs (e.g., diazepam, chlordiazepoxide) suppress defensive burying behavior (13, 31–33, 35), but, paradoxically, increase freezing behavior (32,35). These drugs, therefore, seem to modulate the hierarchy of the rat's defensive coping repertoire (i.e., a transition from an active to a passive mode of responding), which might also be reflected in the hormonal indices.

METHOD

Animals and Housing

Male Wistar rats weighing approximately 280 g on their arrival in the laboratory were used. They were housed individually in clear Plexiglas cages (25 × 25 × 30 cm) on a layer of woodshavings (thickness approximately 3 cm). Cages were placed in a room under conditions of constant temperature (21 ± 2°C) and a fixed 12 hr light/12 hr dark photoperiod (lights on at 7:00 a.m.). Standard laboratory chow (Hope Farms) and water were supplied ad lib. For at least two weeks prior to surgery, rats were handled daily for weighing purposes.

Surgery and Blood Sampling

Under Hypnorm® anesthesia (10 mg/kg fluanisone and 0.2 mg/kg fentanyl) and premedicated with atropine (1 mg/kg) and Valium (5 mg/kg), animals were provided with a silastic cannula (i.d. 0.5 × 0.9 mm tubing; Dow Corning, USA) into the entrance of the right atrium (venae cava) via a jugular venotomy according to the techniques described by Steffens (27). This method allows frequent withdrawal of small amounts of blood without disturbing the animal either behaviorally or physiologically (38). Animals in Experiment 4 were also provided with a silicon cannula (i.d. 0.8 mm; o.d. 1.4 mm) into the antrum wall of the stomach. The outer end of the cannula was extended subcutaneously to emerge at the top of the head and anchored to the skull (28). This catheter allows intragastric drug administration in the freely behaving and undisturbed rat. After surgery, the rats were allowed to recover for at least one week before the start of the experiments. During this period, animals were accustomed to the blood sampling procedure.

Ninety minutes before the start of an experiment the animals were connected to a polyethylene blood sampling tube (length 0.5 m; o.d. 1.45 mm; i.d. 0.75 mm). Animals with a stomach catheter used in Experiment 4 were also connected to a second polyethylene tube allowing drug administration. Blood samples of 0.35 ml were withdrawn for determination of NA, A and CS concentrations. Immediately after each blood sample an equal volume of heparinized (12.5 IU/ml) blood, freshly obtained from a cannulated donor rat, was transfused through the catheter. At the end of the experiment the cannula inside the rat was filled with 0.9% (w/v) NaCl containing 500 IU heparin/ml plus 60% polyvinylpyrrolidone (Merck) and closed with a small polyethylene plug.

Apparatus

Rats were tested in their own home cages. Therefore, no habituation trials and handling of the animals prior to testing were needed, thus preventing disturbance of the neuroendocrine systems under study. In the center of the front wall of the home cage, 2 cm above the upper level of the bedding material, was a small hole through which the shock-prod could be inserted. The shock-prod consisted of a teflon prod (length 6.5 cm; $\phi = 1$ cm) with two uninsulated wires ($\phi = 0.5$ mm) each independently wrapped 25 times around it. The wires were connected to a 1000-volt shock source. Whenever the animal touched both wires simultaneously with some part of its body, an impedance was built up between the two wires and a DC shock (constant current) was delivered to the animal. At the same time, two cumulative counters were triggered; one to register the number of contacts with the prod and the other to monitor the duration of the contacts. The current intensity was 2 mA for the shock condition and a current intensity of 50 nA was used to approach a nonshock condition. During the entire 15-min test the shock circuit was left on, i.e., a "repeated shock-probe procedure" was used (33).

Behavioral Measurements

In addition to the number and duration of contacts with the probe, the animal's behavior was recorded on videotape for 15 min following insertion of the shock-prod into the home cage. From the videotapes, the durations of freezing/immobility and of burying behavior were derived. Freezing was scored when the rat showed an immobilized, crouched posture. Burying behavior was scored when the rat shoveled and/or sprayed bedding material toward or over the prod with its forepaws and/or snout as described by Pinel *et al.* (26).

Experimental Procedure

All experiments were performed in the light period between 1000 and 1300 hours.

Experiment 1. Effects of prod-shock on behavior and plasma CA and CS levels. Ten subjects were randomly divided into a

control nonshock group (n = 5) and an experimental shock (2 mA) group (n = 5). Blood samples were taken at 5 min before shock-prod insertion and at t = 1, 5 and 15 min thereafter. Immediately after the t = 15 min blood sample, the shock probe was retracted and at t = 75 min an additional blood sample was taken.

Experiment 2. Effects of the availability of bedding material on prod shock-induced behavioral and plasma CA and CS responses. Twenty-four subjects were assigned randomly into four groups of six rats each. These groups differed as to the availability of bedding material and the delivery of electric shock. Thus, a factorial design consisting of the following four groups was created: A) bedding material available, prod no-shock; B) bedding material available, prod shock; C) without bedding material, prod no-shock; D) without bedding material, prod shock. One day before testing, bedding material was removed from a rat's home cage and replaced either by new bedding material (group A and B) or by a metal grid floor (group C and D). Blood samples were taken at 15 and 1 min before shock-prod insertion (at t = 0) and at t = 1 and 15 min thereafter. Immediately after the t = 15 min blood sample the shock-prod was retracted and an additional blood sample was taken at t = 75 min.

Experiment 3. Effects of grid-shock on plasma CA and CS concentrations. In order to determine the effect of the source of electric shock on plasma CA and CS responses, ten subjects were exposed to shock administered through the grid floor. Intensity, duration and frequency of the grid-shock were approximately similar to the characteristics of the prod shock in Experiment 2. Like the animals in group C and D from the previous experiment, subjects were housed on a metal grid floor one day before testing. On the experimental day, each individual rat received 3 intermittent constant current scrambled grid shocks (2 mA, 0.02 sec duration every 10 sec). Blood samples were collected 5 min before and 1, 5, 15 and 75 min after the beginning of shock.

Experiment 4. Effects of CDP on the prod shock-induced behavioral and stress hormonal changes. Twenty-four subjects were randomly divided into four groups of 6 animals each. Half of the subjects were tested with bedding material available and the other half without bedding material. Half of each group received 9 mg/kg chlordiazepoxide intragastrically, whereas the other half received vehicle (0.9% NaCl). Immediately after taking the first baseline blood sample (at t = −60 min), drug or vehicle solutions (2 ml/kg) were slowly (10 sec) infused via the intragastric cannula. Blood samples were collected 1 min before shock-prod insertion (t = 0) and at t = 1 and 15 min thereafter. Sixty minutes after shock-probe retraction (at t = 75 min) an additional blood sample was taken.

Chemical Determinations

Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 10 μl heparin solution (500 IU/ml) as anticoagulant. For the determination of plasma catecholamine contents, an aliquot of 250 μl transferred blood was then rapidly pipetted into chilled tubes containing 10 μl of a solution of 25 mg/ml disodium EDTA and 27.5 mg/ml reduced glutathione in order to prevent CA degradation. The remaining 100 μl blood was used for the CS assay. After centrifugation (4000 × g for 10 min at 4°C), supernatants were removed and stored at −30°C.

The concentrations of NA and A were measured in duplicate in 20 μl perchloric acid-deproteinized plasma according to a radioenzymatic COMT-procedure (36). The CAs were converted into their [^{3}H]-methoxy derivatives by incubation with S-adenosyl-L-[methyl-^{3}H]methionine (80 Ci/mmol; NEN Chemicals) in the presence of catechol-O-methyltranferase. Labeled products were isolated by organic extraction and paper chromatography. After elution of labeled products, activity was counted in a liquid scintillation analyzer (Philips, The Netherlands). CA concentrations were calculated from net DPM values for samples and internal standards and were expressed as pg/ml. The intra- and interassay variabilities were less than 10% and 15%, respectively. The sensitivity of the assay (amount corresponding to twice the blank) was 1 pg for both NA and A.

Plasma CS concentrations were determined in duplicate according to a competitive protein-binding method (24). Corticosterone was extracted with dichloromethane for 25 μl samples of plasma. The dried residue was incubated with a corticosteroid-binding globulin tracer solution {0.1% plasma from adrenalectomized female rats containing [1,2-^{3}H]-corticosterone (40–50 Ci/mmol; NEN Chemicals) as tracer}. Unbound steroid was removed using dextran-coated charcoal. Standard CS was supplied by Sigma. The intra- and interassay coefficients of variation were less than 10%. Fifty percent displacement of tracer steroid was obtained at a concentration of 20 ± 2 μg/dl.

Statistical Analyses

Behavioral data were evaluated by use of one- or two-way analyses of variance (ANOVA) with experimental condition(s) as between-subjects factor(s), i.e., Experiment 1: shock condition (shock-no shock); Experiment 2: bedding condition (bedding-no bedding) as factor 1 and shock condition (shock-no shock) as factor 2; Experiment 4: bedding condition (bedding-no bedding) as factor 1 and drug condition (drug-vehicle) as factor 2. The time patterns of each hormone were evaluated using a two-way (Experiment 1) or three-way (Experiments 2 and 4) ANOVA with experimental condition(s) as between-subjects factor(s) (as indicated above) and sampling time as repeated measures within-subject factor (5 levels). The hormonal patterns of Experiment 3 were analyzed using a one-way ANOVA with sampling time as repeated measures within-subject factor (5 levels). The multivariate model was used for the repeated measures factor (6,10). Further analyses were made by *t*-tests to determine the source of detected significances in the ANOVAs. The criterion of significance was set at $p<0.05$.

RESULTS

Experiment 1

It is apparent from Fig. 1 that the exploration of a prod inserted into a rat's home cage, quantified as the number and duration of times the animal makes physical contact with it, was reduced when the prod was electrified. Furthermore, shocked rats spent considerably more time freezing and burying the prod than their respective nonshocked controls did. These behavioral results were confirmed by the significant main effects of shock condition in the corresponding ANOVAs; prod contacts: $F(1,8) = 15.4$; contact time: $F(1,8) = 33.8$; freezing: $F(1,8) = 10.1$; burying: $F(1,8) = 54.7$.

Figure 2 shows the mean time course of changes in plasma CS, A and NA concentrations in response to electrified and nonelectrified shock-prod exposure. ANOVA on the CS values yielded only a significant main effect of sampling time, $F(4,5) = 5.9$. Neither the main effect of shock nor the interaction shock × sampling time reached statistical significance. Further analyses revealed that there was a comparable small increase of CS for both the shocked and nonshocked animals at t = 15 min during prod exposure.

ANOVA on the A values showed a significant main effect of sampling time, $F(4,5) = 42.5$, and of the interaction shock × sampling time, $F(4,5) = 13.5$. The nonshocked subjects did not

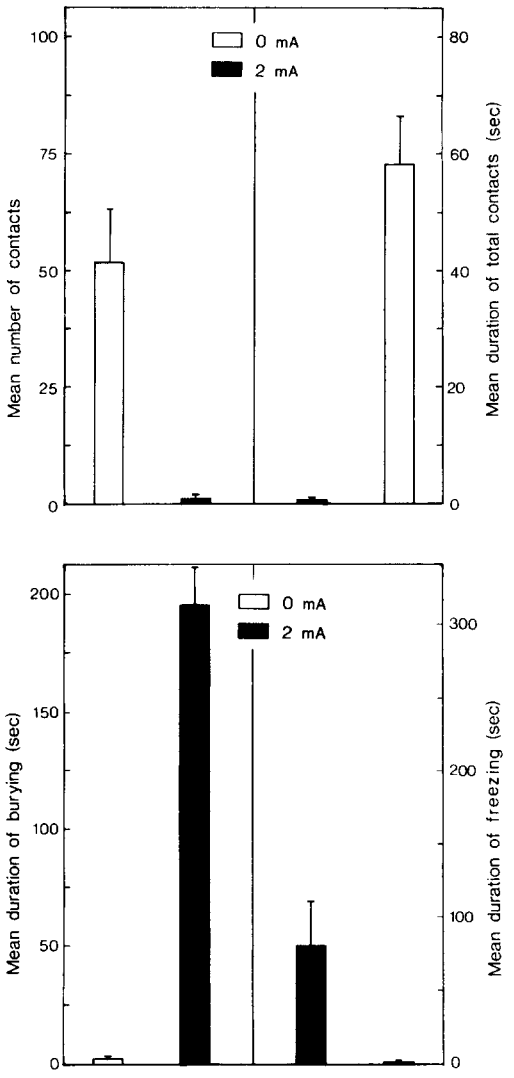

FIG. 1. Mean (±SEM) number and duration of prod contacts (upper panel) as well as mean (±SEM) duration of burying and freezing behavior (lower panel) by shocked (2 mA) and nonshocked (0 mA) subjects (n = 5 for each group).

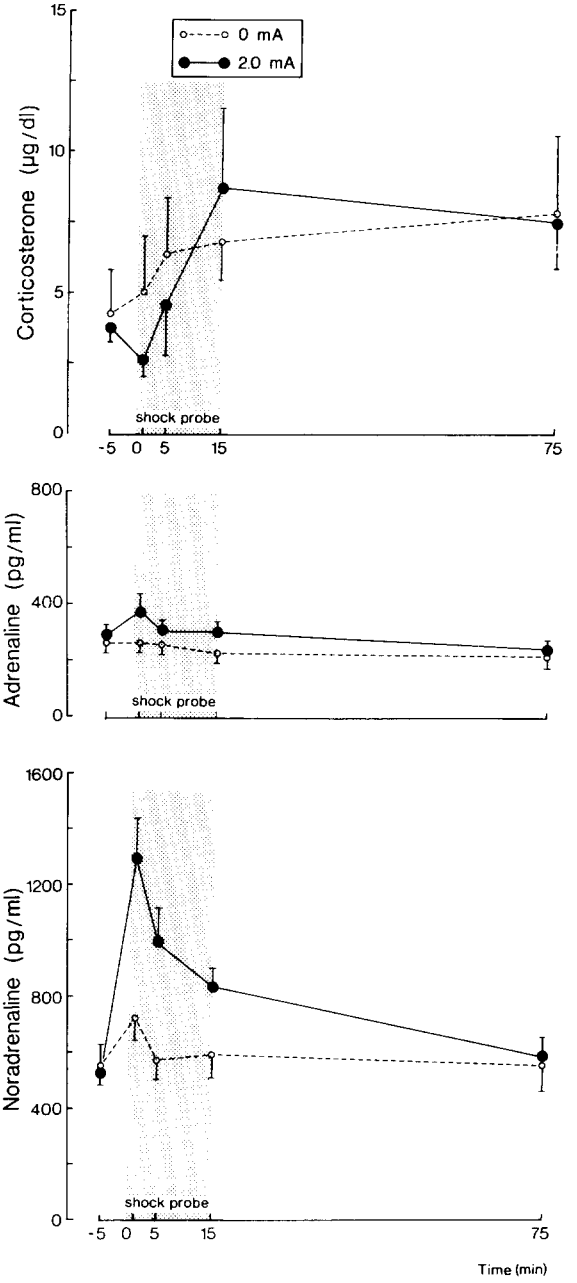

FIG. 2. Plasma corticosterone, adrenaline and noradrenaline concentrations in rats before, during and after 15-min exposure to an electrified (2 mA) and nonelectrified (0 mA) shock-prod in the home cage. Data are expressed as mean ± SEM for 5 rats per condition.

show a change in A levels during the shock-prod session, whereas a slight but significant increase at t = 1 and t = 15 min was found in the shocked animals.

For the NA values, ANOVA yielded significant main effects of shock condition, $F(1,8) = 8.24$, sampling time, $F(4,5) = 10.7$, as well as a reliable shock × sampling time interaction, $F(4,5) = 5.34$. Subsequent analyses indicated a small increase of NA concentrations 1 min after probe insertion for the nonshocked group of rats. In the shocked group of animals, the initial prod-induced NA elevation was much higher and remained significantly elevated during the 15-min shock-probe session.

Experiment 2

Figure 3 shows the effect of the presence and absence of bedding material on the behavioral measures for shocked and nonshocked subjects. As observed in Experiment 1, shocked rats showed a reduced exploration of the probe, irrespective of whether bedding material was available or not. A two-way ANOVA confirmed this observation by yielding only significant main effects of shock for the number of prod contacts, $F(1,21) = 50.1$, and for the duration of prod contacts, $F(1,21) = 34.8$. No significant main effects of bedding or bedding × shock interaction effects were found. As was the case in Experiment 1, shocked rats with bedding material available spent significantly more time burying the prod than the nonshocked rats, $F(1,21) = 60.5$. However, shocked rats without the burying option (no bedding material available) engaged in more freezing than the shocked animals in an

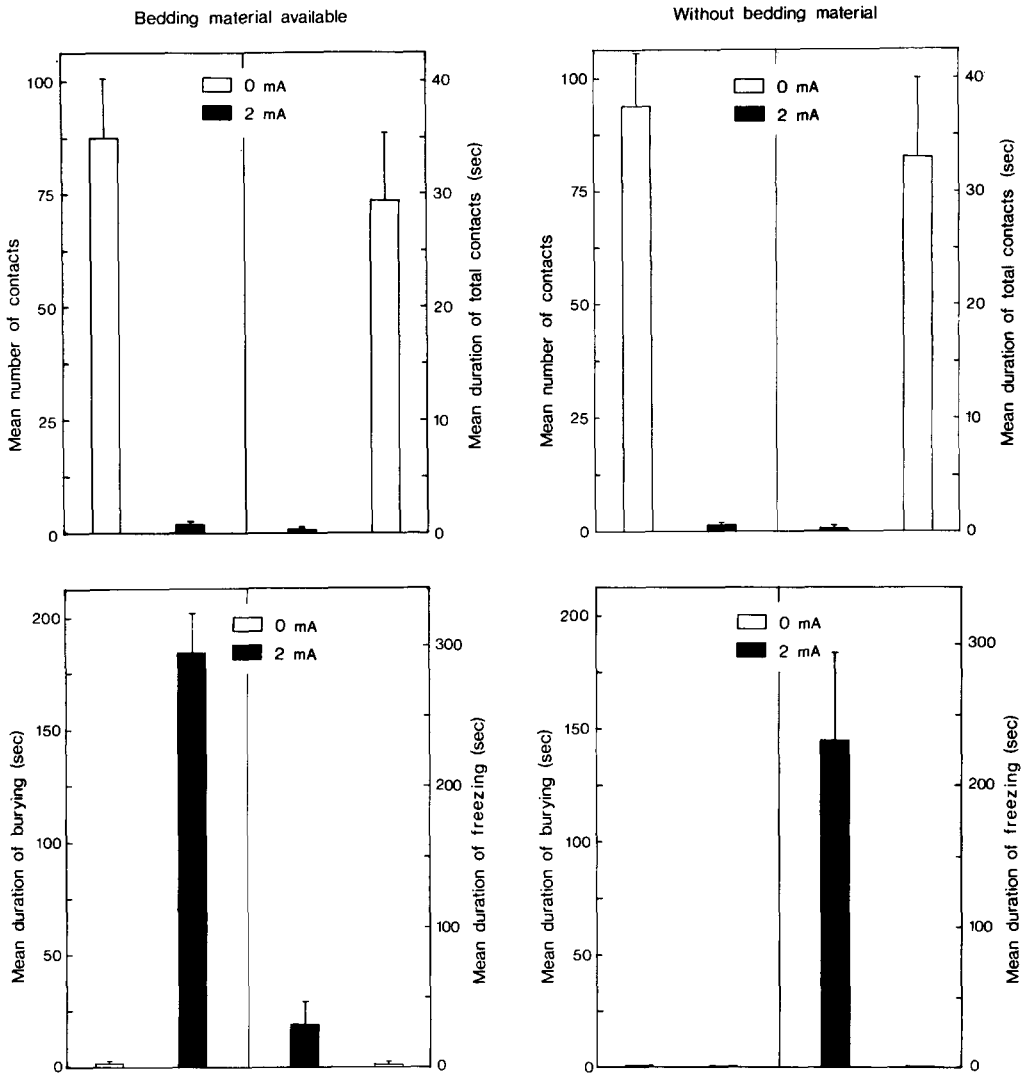

FIG. 3. Mean (±SEM) number and duration of prod contacts (upper panels) as well as mean (±SEM) duration of freezing and burying behavior (lower panels) by shocked (2 mA) and nonshocked (0 mA) subjects tested with (left panels) or without (right panels) bedding material available in their home cages (n = 6 per condition).

environment with bedding material on the floor. These findings were confirmed in the ANOVA which revealed significant effects of bedding, $F(1,21) = 13.3$, shock, $F(1,21) = 21.5$, as well as a significant bedding × shock interaction, $F(1,21) = 13.4$.

Figure 4 shows the effect of the availability of bedding material on the dynamics of plasma CS, A and NA secretion in response to electrified and nonelectrified shock-probe exposure. Three-way ANOVA on the CS values yielded a significant main effect of sampling time, $F(3,19) = 37.2$, a significant shock × sampling time interaction effect, $F(3,19) = 6.6$, as well as an overall bedding × shock × sampling time interaction effect, $F(3,19) = 7.5$. In all four groups of animals, a significant increase of CS was observed at $t = 15$ min after probe insertion. But this increase was significantly larger in the shocked group of animals tested in the grid-floor chamber without bedding material.

ANOVA on the A values indicated significant main effects of bedding, $F(1,21) = 4.4$, shock, $F(1,21) = 18.2$, and sampling time, $F(3,19) = 19.2$. Sampling time interacted significantly with shock condition, $F(3,19) = 11.6$. Shocked and nonshocked groups of rats were analyzed separately, with bedding condition and sampling time as main factors. In the nonshocked rats the A levels did not change over the sampling period, whereas the A concentrations in the shocked rats increased during the shock-probe session as observed in Experiment 1 [time: $F(3,9) = 19.1$]. However, the A elevation of the shocked rats without bedding material was higher than the corresponding values of the shocked rats with bedding material available [time × bedding interaction, $F(3,8) = 4.62$].

ANOVA on the NA data showed significant main effects of shock, $F(1,21) = 13.8$, and sampling time, $F(3,19) = 29.2$, as well as significant interaction effects of shock × sampling time, $F(3,19) = 11.2$, and bedding × shock × sampling time, $F(3,19) = 5.25$. Subsequent analyses indicated a small increase of NA concentrations at $t = 1$ min after probe insertion for the unshocked groups of rats. This probe-induced NA elevation was significantly higher in the shocked groups of animals as compared to the unshocked groups. After this initial increase, the NA contents returned to basal level, except for the shocked group of rats in the bedding-floor cages where it remained significantly elevated up to

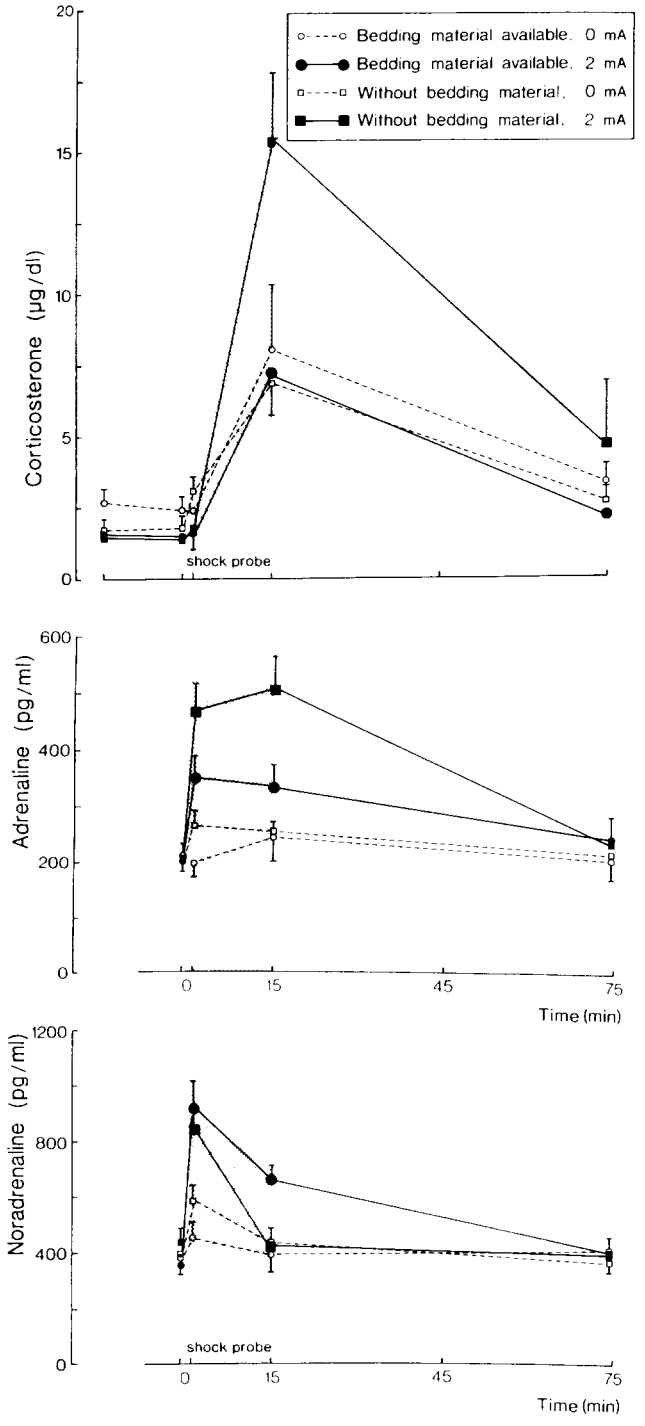

FIG. 4. Plasma corticosterone, adrenaline and noradrenaline concentrations before, during and after 15-min exposure to an electrified (closed symbols) or nonelectrified (open symbols) shock-prod within rat's home cage either with (circles) or without (squares) bedding material on the floor. Data are expressed as means ± SEM for 6 rats per condition.

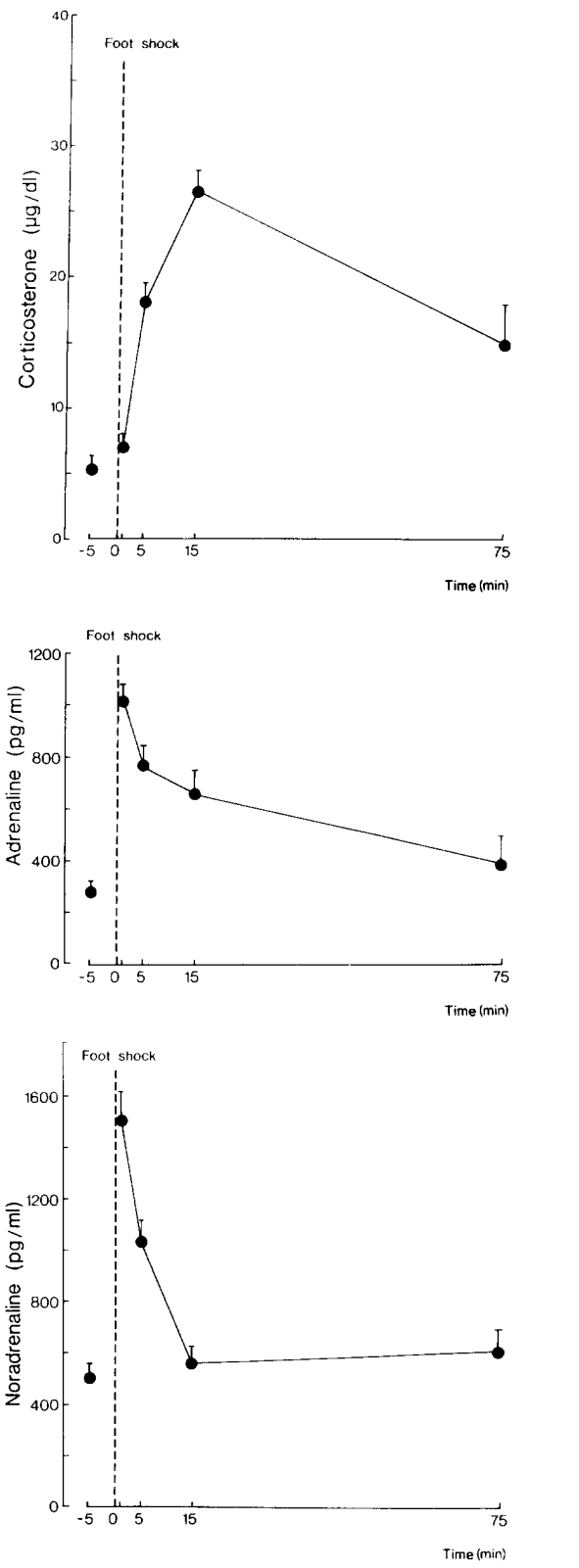

FIG. 5. Mean (± SEM) plasma corticosterone, adrenaline and noradrenaline concentrations in rats before (−5 min) and 1, 5, 15 and 75 min after delivery of 3 scrambled, constant current grid-shocks (2 mA, 0.02 sec duration every 10 sec) (n = 10).

t = 15 min as compared to either baseline values or the corresponding values of the group of subjects for which bedding material was available.

Experiment 3

During and shortly after delivery of grid-shock, rats were

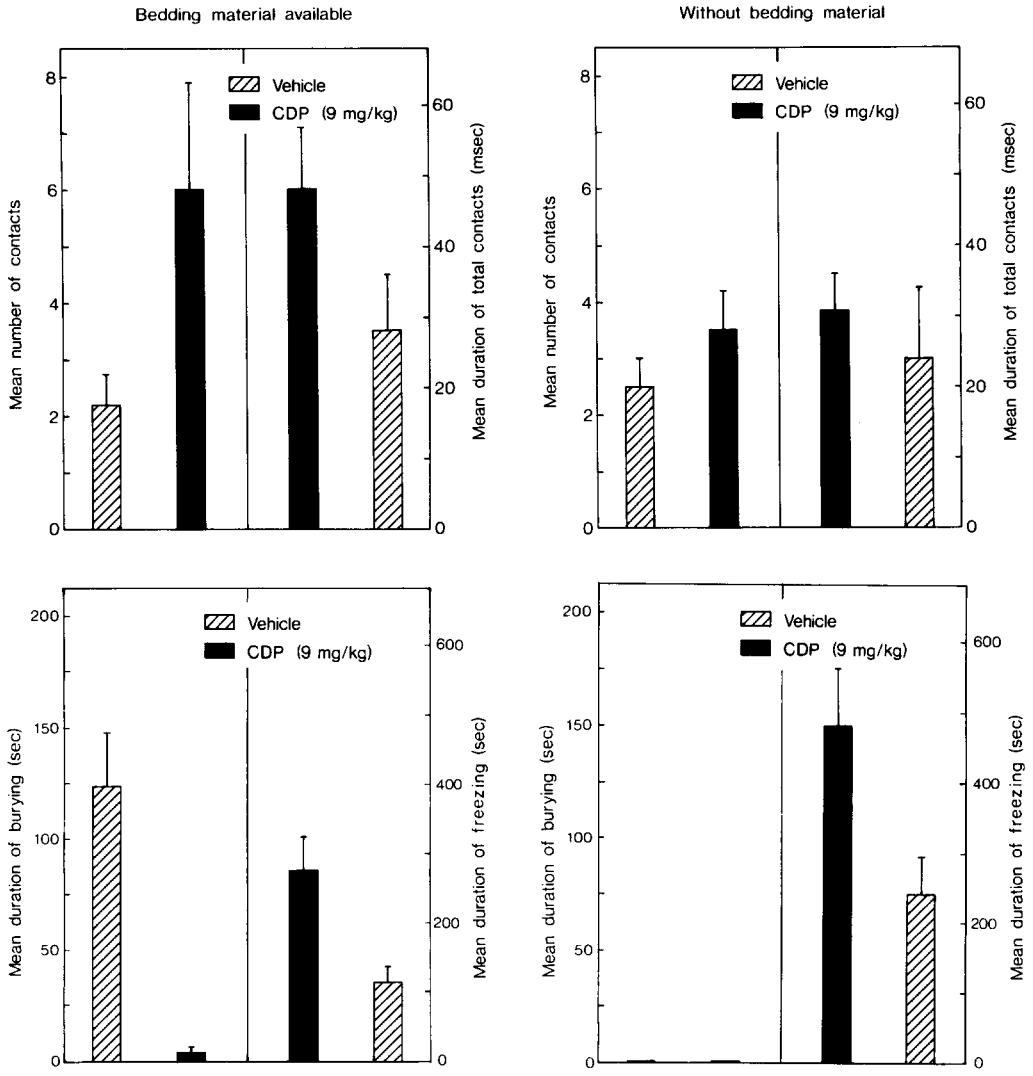

FIG. 6. Mean (± SEM) number and duration of electrified prod contacts (upper panels) as well as mean (± SEM) duration of freezing and burying behavior (lower panels) by vehicle- and chloridazepoxide (CDP)-treated rats tested in either the presence (left panels) or absence (right panels) of bedding material (n = 6 for each condition).

active and attempted to escape by repeated rearing and jumping, whereafter they assumed a passive, immobile posture and remained very alert.

Figure 5 represents the mean time course of changes in plasma CS, A and NA concentrations in response to foot-shock. One-way ANOVA revealed significant effects of time for all three hormones; CS: $F(4,6) = 35.2$; A: $F(4,6) = 25.4$; NA: $F(4,6) = 32.8$. In response to shock, plasma A and NA contents increased immediately and peaked at the t = 1 min sample, whereafter they declined somewhat at t = 5 min. Subsequently, the NA concentration returned to basal level at t = 15 min. However, at this timepoint A content still remained significantly elevated. Plasma A returned to basal value at t = 75 min. In contrast to this momentary reaction of plasma CA, the rise in plasma CS concentrations was slower in onset, peaked later (at t = 15 min) and was slower in decline (still significantly elevated at t = 75 min). The grid-floor foot-shock-induced CS, A and NA increases (peak values) were considerably larger in comparison with the prod-shock-induced hormonal responses of rats tested in grid-floor cages (Experiment 2) [CS: $t(14) = 4.01$; NA: $t(14) = 3.77$; A: $t(14) = 5.52$].

Experiment 4

Figure 6 shows that 9 mg/kg of CDP increased the number of contacts with the electrified prod, $F(1,20) = 5.08$, and the amount of time spent freezing, $F(1,20) = 14.2$, regardless of whether bedding material was available or not. Furthermore, the amount of burying behavior displayed by the CDP-treated rats was significantly lower than the amount displayed by vehicle-treated rats, $F(1,20) = 39.0$. As was the case in Experiment 2, shocked rats in the grid-floor cages spent significantly more time freezing than shocked animals in the bedding-floor cages, $F(1,20) = 9.08$. But regardless of whether bedding material was available or not, CDP-treated animals froze longer than vehicle-treated animals.

The effects of CDP treatment on the electrified shock-prod-induced CS, A and NA responses of rats tested either in bedding-floor cages or in grid-floor cages are shown in Fig. 7. ANOVA on the CS data revealed only a significant main effect of sampling time, $F(4,16) = 19.5$. But sampling time interacted significantly with both bedding condition, $F(4,16) = 4.64$, and drug condition, $F(4,16) = 18.0$. Subsequent analyses indicated that administration

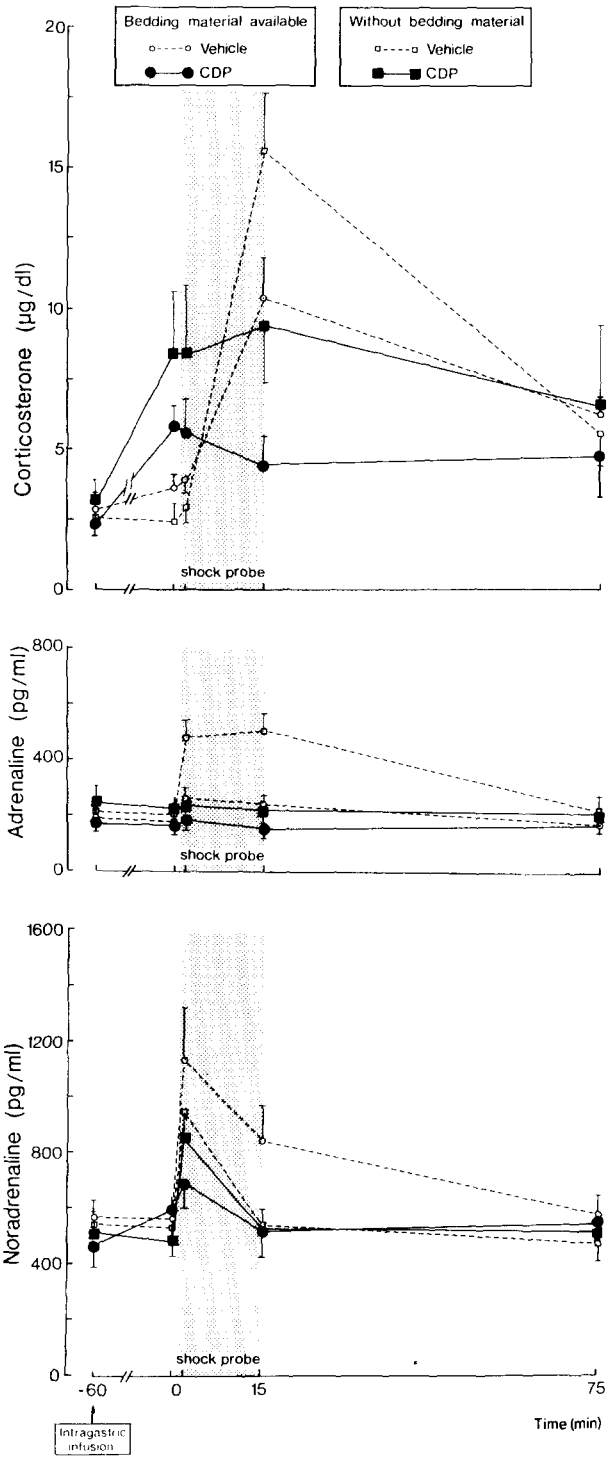

FIG. 7. Plasma corticosterone, adrenaline and noradrenaline concentrations before, during and after 15-min exposure to an electrified shock-prod in vehicle- (open symbols) and chlordiazepoxide (closed symbols)-treated rats tested either with (circles) or without (squares) bedding material on the floor. Data are expressed as mean ± SEM for 6 rats per condition.

of CDP produced a moderate increase of basal plasma CS release before the insertion of the shock-prod, and prevented a further CS increase induced by electrified prod exposure. As was the case in Experiment 2, the prod-induced CS increase at t = 15 min for vehicle-treated rats tested in grid-floor cages was significantly larger as compared to the corresponding value for vehicle-treated rats tested in the bedding-floor cages.

ANOVA on the A values indicated significant main effects of bedding, $F(1,20) = 12.6$, drug, $F(1,20) = 7.91$, and sampling time, $F(4,17) = 9.76$. Sampling time interacted significantly with bedding condition, $F(4,17) = 3.84$, and drug condition, $F(4,17) = 9.37$. Similarly to the results of Experiment 2, the prod shock-induced A elevation (at t = 1 and 15 min) of vehicle-treated animals in grid-floor cages was significantly higher in comparison with the A increase of vehicle-treated subjects in bedding-floor cages. In both the bedding- and grid-floor chambers, drugged animals showed no plasma A elevations in response to electrified prod exposure.

ANOVA on the NA data yielded only a significant main effect of sampling time, $F(4,17) = 12.0$. The overall interaction between bedding, drug and time just reached significance, $F(4,17) = 2.96$. As compared with vehicle-treated animals, the shock-prod-induced NA increase was attenuated in CDP-treated rats tested on a bedding-floor. In the grid-floor environment, however, drugged rats showed a similar NA increase at t = 1 min compared to vehicle-treated rats. As observed in Experiment 2, the plasma NA contents at t = 15 min of vehicle-treated rats in bedding-floor cages was significantly higher compared to vehicle-treated rats in grid-floor cages.

DISCUSSION

The results of the present four experiments illustrate that the shock-prod defensive burying/freezing paradigm served well as an experimental setting for assessing the neuroendocrine correlates of active and passive avoidance behavior in rats. Consistent with previous reports (26, 32, 34), the data clearly show that, depending on the availability of bedding material, rats cope with a well-localized source of aversive stimulation (an electrified prod) within their home cage by means of an active (i.e., burying the prod in the presence of bedding) or a passive (i.e., freezing when no bedding is available) form of avoidance responding.

Although actively and passively coping rats received similar amounts of electric shock through the stationary prod, their profiles of changes in plasma NA, A and CS concentrations differed substantially from each other. Concentrations of NA and A in circulating blood reflect neurosympathetic and adrenomedullary outflow respectively, whereas plasma CS concentrations serve as an indicator of pituitary-adrenocortical activity (16, 19, 29). Burying the shock-prod is accompanied by an increased activation of both the neurosympathetic and adrenomedullary component of the sympathoadrenal system as indicated by elevated plasma NA and A levels. However, the neurosympathetic outflow during active coping is relatively higher than the adrenomedullary output as reflected by an increased NA/A ratio (see Table 1). Freezing in locations away from the prod is associated with pituitary-adrenocortical and sympathoadrenal activation, as indicated by elevations in CS, NA and A concentrations. However, during the passive coping situation the adrenomedullary output is relatively higher than the neurosympathetic outflow as reflected by a decreased NA/A ratio (Table 1). A decrease in the ratio of NA to A was also observed in rats shocked through the grid-floor (Experiment 3; Table 1) and displaying passive, immobile behavior after initial disorganized and unsuccessful active escape at-

TABLE 1

CHANGES IN THE RATIO OF NORADRENALINE TO ADRENALINE OVER TIME IN NONSHOCKED (0 mA) AND PROD-SHOCKED (2 mA) RATS WITH OR WITHOUT BEDDING MATERIAL AVAILABLE AS WELL AS IN GRID-SHOCKED (2 mA) RATS

Time (min)	With Bedding		Without Bedding		Grid Shock
	0 mA	2 mA	0 mA	2 mA	2 mA
−5/−1	2.0 ± 0.08	2.3 ± 0.19	2.0 ± 0.17	2.2 ± 0.14	1.8 ± 0.13
+1	2.4 ± 0.16*	3.5 ± 0.30*†‡	2.4 ± 0.36	2.0 ± 0.22	1.6 ± 0.25
+15	2.2 ± 0.22	2.9 ± 0.23*‡	1.8 ± 0.13	0.9 ± 0.08*†	0.9 ± 0.12*†
+75	2.2 ± 0.16	2.5 ± 0.30	1.9 ± 0.22	1.9 ± 0.13	2.1 ± 0.40
n	11	17	6	12	10

Values are expressed as mean ± SEM; n = number of animals.

Two-way ANOVA on the NA/A values revealed significant effects of the main factors group, $F(4,52) = 6.74$, and time, $F(3,50) = 14.7$, as well as a significant group by time interaction effect, $F(12,156) = 4.78$. Results of subsequent analysis are indicated by the following symbols: *$p<0.05$ as compared to the basal value of the corresponding group. †$p<0.05$ as compared to the value of the respective nonshocked group. ‡$p<0.05$ as compared to the value of the corresponding prod-shocked group without bedding and grid-shocked group.

tempts. It is suggested, therefore, that the prod-shock without bedding condition and the foot-shock condition constitute a noncoping situation for the rat in which the resulting passive avoidance behavior is accompanied by a relatively high pituitary-adrenocortical and adrenomedullary activation. On the other hand, active avoidance behavior in the prod-shock bedding condition is accompanied by a relatively high sympathoadrenal activation. These observations, obtained within a nonsocial context, reinforce the stress concept by Henry and colleagues (17,18) that different behavioral coping patterns to a challenge are associated with different patterns of neurosympathetic, adrenomedullary and adrenocortical activation. Further, the data provide empirical support for the suggestion (16, 22, 40) that sympathetic nervous activity, indicated by plasma NA, is principally related to situations involving actual skeletal muscle exertion, regardless of the amount of emotional stress, fear and/or anxiety, whereas adrenomedullary and pituitary-adrenocortical stimulation occurs mainly during emotional stress, fearful or anxiety-provoking conditions characterized by limited, no or abolished coping capabilities. Thus, the NA/A ratios in circulating blood reflect an animal's physical activity relative to its emotional arousal state (fear, anxiety) induced by the environmental challenge. In addition, the changes in NA and A cannot be explained on basis of changes in the activity of the pituitary-adrenocortical axis, since elevated CS concentrations are not uniformly accompanied by raised CA contents and because increases in plasma NA and A concentrations precede a significant activation of the pituitary-adrenocortical axis.

The view that plasma A and CS concentrations are hormonal indices of emotional stress is further supported by the observation that pretreatment with an anxiolytic dose of the benzodiazepine chlordiazepoxide completely abolished the prod-shock stress-induced A and CS increase both in the bedding-floor and the grid-floor condition. The suppression of burying in CDP-treated rats was accompanied by a reduction in the plasma NA rise of these animals as well, and therefore reinforced the notion of a link between physical activity and NA release.

A number of previous studies have shown that (emotional) stress-induced glucocorticoid elevations can be blocked or attenuated by pretreatment with relatively low doses of BDZ's (20, 21, 23, 25, 30). In contrast, there appears to be little information about anxiolytic drug effects on exercise or emotional stress-induced sympathoadrenal release of catecholamines in the rat. Only one study has reported that a low (nonsedating) dose of midazolam reduced the foot-shock stress-induced plasma A but not the NA response, whereas a higher (sedative/ataxic) dose attenuated both the NA and A stress response (9). For this reason, a number of investigators have considered inhibition of stress-related glucocorticoid and/or adrenaline secretion to be predictive of or at least closely related to anxiolytic drug action at a behavioral level (9, 20, 30). The suppression of burying responses in the shock-prod paradigm has been employed as a behavioral measure to detect and/or screen anxiolytic property of drugs. Prototypical antianxiety agents like diazepam and chlordiazepoxide reliably decrease the duration and number of burying behavior in a dose-related manner (1, 3, 32, 33, 35). However, at the same time these drugs enhance freezing behavior (32,35). The present study replicated this anxiolytic-induced shift in the coping style of rats. Because both burying and freezing responses are characterized as behavioral indices of the animal's fear and/or anxiety (5,39), the nonuniform suppression of these behavioral indices by a prototypical anxiolytic makes the interpretation of drug effects on fear and anxiety in this paradigm problematic. Moreover, it has been found that increasing fear, as a result of prior exposure to uncontrollable electric foot-shock and/or the presence of conspecific stress odors, potentiates freezing and disrupts burying behavior (39), indicating a complex relationship between levels of fear and the occurrence of burying and freezing behavior in the rat. The results of the present study, however, show that the paradoxical CDP-induced increase in freezing behavior is accompanied by completely abolished plasma CS and A responses, indicating that fear and/or anxiety state is reduced after CDP treatment. If the CS and A responses are to be considered true indicators of anxiety states, then in this paradigm freezing behavior cannot be an indicator of changes in fear and anxiety. One might assume that after administration of CDP the animal experiences the situation as less aversive and therefore needs considerably less exploratory behavior and other preventive actions. Thereby it seemingly exhibits more freezing. It can be reasoned, therefore, that the combined assessment of behavioral and humoral indices of the animals' fear

and/or anxiety state within the same experimental setting may facilitate the interpretation of anxiolytic drug actions.

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